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# DEVELOPMENT OF VIROLOGY AS AN INDEPENDENT SCIENCE*

BY

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I welcome this opportunity for acknowledging the honour of being invited as a visitor to this great University and of being asked to give the first Pfizer Lectures in Virology. The experience of residing here for an extended period of time, of enjoying the intellectual stimulation of my colleagues, and of working in the laboratories of the Institute of Virology have all given me unprecedented pleasure. I wish to thank the Pfizer company for their generosity and foresight in making this lectureship possible and I hope that this will be the first of a long series.

The evaluation of virology as a separate science is an appropriate topic for the opening general lecture of a series sponsored by the Institute of Virology and it necessitates a brief historical review, with special emphasis on those forces which in recent years have helped to unify the several branches. In this review I shall try to make it clear that virology can make its fullest contribution to biology and medicine only if it is treated and studied as an independent discipline and is allowed to be fully responsive to its own internal needs. In emphasizing the importance of breaking away from the traditional link to medicine, I have animal virology mainly in mind, and I am fully aware that this shift runs counter to the instincts of many of my colleagues.

## History of Virology

The history of virology is a complex story of the independent development of several different branches, each involving a different group of hosts: flowering plants, vertebrates, insects, and bacteria. These branches arose and developed separately and have become interdependent only recently. The overlong period of isolation was, in part, a consequence of the very different hosts involved, but the separation of the branches was widely supported by the virologists themselves, partly because the prominent systemic symptoms of virus infection obscured a conception of common events at the cellular level.

There was no serious attempt to integrate the findings in the various virus fields until Luria's textbook on *General Virology* appeared in 1953. Later texts on animal virology *(Viral and Rickettsial Infections of Man,* 1959; *Principles of Animal Virology,* 1960) treat the general approach to virology in only the most superficial way. Even as late as 1956 a prominent animal virologist, writing about virus multiplication, was moved to say that resemblances between T2 and influenza viruses were "superficial and inappropriate for serious generalization." To-day it is more appropriate to emphasize that it is the differences between animal and bacterial viruses which are superficial, and the intention in this lecture is to emphasize the importance of generalization in this field.

Initially, virology was an offshoot of pathological bacteriology, and this is a tie which has affected its entire development. Although virus diseases have been known for centuries, the first virus, tobacco mosaic virus, was described in 1892 by Iwanowski. It is not surprising that neither he nor his contemporaries fully understood the meaning of an infective particle passing through an earthenware filter, and even in later years Iwanowski's fame as the father of virology was appreciated abroad more than at home, and it was not until the middle 'fifties that one of the many virological institutes in Moscow was named after him. The first animal virus (foot-and-mouth disease of cattle) was described six years later, in 1898. From this time on the number of filterable agents increased and, understandably, there was a gradual acceptance of the idea that they formed a distinct group and were not merely bacteria which had difficult growth requirements.

The rules for inclusion of an organism within the virus group developed slowly and empirically. The first criterion, that of size, was a governing factor from the beginning, and the criterion of filterability, though arbitrary, was unexpectedly successful in delimiting this group without much ambiguity. The fact of obligate intracellular parasitism, the high specificity for the host cell, and the frequency of a necrotic response after infection were all features common to all types of viruses from the beginning, but they were not generally considered of sufficient weight to suggest strongly that there was any real unity in the group, except by accident. We shall leave further consideration of this problem until we have reviewed the history of the other branches.

## Plant Virology

Plant virology has usually been a branch of plant pathology, and great emphasis has always been placed on the discovery of new viruses, description of their mode of spread, and investigation of prophylactic measures. Nearly all plant viruses produce undesirable effects in their hosts, and the preponderance of attention has been placed on those agents which cause an economic loss, as is illustrated by the fact that nearly all of the viruses which have been described are parasitic on plants of commercial importance, such as tobacco, potatoes, peaches, spinach, and others. In spite of this

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intensely practical orientation, plant virus research has often been productive of new concepts which have been influential throughout all virology.

Not only were plant viruses the first viruses to be discovered and the first to be crystallized, but they were the first to evoke an interference effect in the laboratory. They were the first viruses in which the details of particle structure were clarified at a molecular level, the first viruses with which infection was initiated by nucleic acid alone, and the first in which mutation was induced *in vitro*. Cloning of plant viruses was accomplished by a plaque technique long before a similar method was applied to animal viruses.

The scope of plant virology began to widen in the early 1930's when the crystallization of tobacco mosaic virus (T.M.V.) attracted the eye of the biochemist, since here was an infective agent which could be obtained in pure form and in gramme quantities.

The careful biochemical and biophysical work which followed was most important in establishing a general type of structure for the most elementary type of virus particle. In the case of T.M.V. this consisted of a nucleic acid helix embedded in a helix of a repeating single type of protein. In the case of some other plant viruses the nucleic acid is packed in a roughly spherical shape but is covered by protein units in such a way that polyhedra of great regularity are formed. Both of these structures have since been found among animal viruses, by electron microscopy with the phosphotungstic-acid staining technique. These striking morphological similarities provide the most satisfying evidence obtained thus far for the fundamental unity of the virus world.

The discovery of the infectivity of T.M.V. nucleic acid was followed almost immediately by similar demonstrations with animal viruses and, later, with bacterial agents. This has affirmed the importance of the nucleic acid core as the repository of all the essential information for replication of the total virus. It is also apparent from T.M.V. work that one of the main functions of the coat is to preserve the core in the extracellular state, and this is probably one of its functions for all viruses. Whether or not all virus coats play the same part in regard to attachment is not entirely clear as yet.

The interest in plant viruses continues from the academic standpoint and has extended now so that one phase of T.M.V. work involves the general problem of protein synthesis. The induction of mutants in T.M.V. with nitrous acid was rapidly followed by similar demonstrations with poliomyelitis and Newcastle disease virus, as well as with phage.

The academic approach to plant virology has been most fruitful and it has been very important in creating a fundamental picture of the virus particle. Its impact on animal virology in other respects has been less, and this may well be due to necessarily different methodologies. It requires a very large amount of virus to infect a plant, and the reasons for this and the details of the infective process are poorly understood. There is no way of studying the infection at the cellular level. The lack of a usable tissue-culture system and the necessity of working with whole plants or whole leaves have made it difficult to study in detail such varied phenomena as penetration, eclipse, mixed infection, recombination, and other reactions which are the centre of interest in other fields.

## Animal Virology

Now let us consider the development of the science of the vertebrate viruses or of veterinary and medical virology. At the outset animal viruses were lumped with pathogenic bacteria, and for a great many years most animal virus research was carried out either in medical schools or in organizations closely allied with the health sciences. A large proportion of virologists have been primarily trained in medicine, and often they have had no formal education in microbiology apart from that given in medical school. The general attitude has been that virology, lacking a well-developed body of technique, was a discipline which could be easily mastered in a couple of years by a medical-school graduate, provided he had no aversion for the subject.

Inevitably with this background, the attention of investigators was focused on viruses as agents of disease. The pressure in pathology and medicine was for the isolation of new agents from diseases of unknown aetiology, and for efforts to cure or prevent diseases of known virus aetiology. Relatively little emphasis was placed on the virus particle itself or on virus infection at a cellular level.

For many years the progress of animal virology was hampered by the lack of good techniques. Since viruses may have very specific tropisms, the discovery of suitable hosts always remained a central problem. The rabbit, dog, and monkey of the earlier years were rapidly replaced in favour by the mouse, especially when the efficacy of intracerebral injection was discovered. The introduction of each new host was accompanied by the isolation of some new agents; sometimes these were agents latent in the host. The discovery of the high susceptibility of ferrets to influenza by a natural route created a wide but transient interest in unusual animals as potential hosts for stubborn viruses.

The next step was the widespread use of the chick embryo. This was important because it marked a departure from the concept that the laboratory host should provide a reasonable facsimile of the human infection. While the chick embryo had many advantages, such as broad susceptibility, it was still a very complicated host. Therefore the next step, the use of animal cells cultivated *in vitro*, was by far the most important advance, not only because it fostered a great increase in the number of viruses cultivable but because it eliminated a great many of the complications of working in the whole animal and fostered investigation of infection at a cellular level. Actually the techniques of tissue culture are quite old, and this source of host material might well have been developed much earlier if virologists had not been so preoccupied with models of the clinical disease.

In general, the discovery of new agents was followed by a fairly regular pattern of development: adaptation of the virus to new and convenient hosts, hopefully accompanied by high levels of virus production; establishment and evaluation of clinical diagnostic tests, and the application of such tests to the epidemiology of the disease; and, finally, the study of disease prevention, which usually took several forms—vaccination with killed virus, vaccination with live but attenuated virus, and environmental or vector control.

The magnitude of the effort in applied virology has thus always been much greater than that devoted to a

long-range approach, and there seemed to be adequate reason for satisfaction with this emphasis because the application of rather empirical methods to public health problems has actually worked very well. Smallpox, urban yellow fever, and poliomyelitis are all good examples of diseases brought under control by these methods.

It is a sort of paradox that the most sophisticated solution to the prophylaxis of a virus disease was the use of live attenuated virus for protection against smallpox, and was due to Jenner in the eighteenth century, long before the era of microbiology. Urban yellow fever was well controlled by an attack on the vector, *Aedes aegypti*, long before the dispute over the aetiological agent had been settled and the virus isolated. Finally, there is the recent example of poliomyelitis, against which both killed and attenuated viruses have been used. No new principles were involved and success awaited the modern tissue-culture era which provided clean high-titre virus preparations and the possibility of antibody titrations on a large scale without the use of monkeys.

We shall momentarily leave animal virology at this point, to return to it again later after considering the history of bacterial virology, which should provide better perspective concerning the more recent history.

## Bacteriophage

We turn our attention now to a most important event in the history of our science, the discovery of bacterial viruses or bacteriophage. This was due to a British bacteriologist, Twort, who in 1915 described a disease of staphylococci. Among the hypotheses he considered was that of a viral disease of bacteria. This paper, though provocative and perceptive, passed unnoticed, and Twort did not further pursue the matter, possibly because it was wartime.

The same reaction was independently discovered by a Canadian, d'Herelle, who was looking for a synergistic action between a virus and a bacterium in dysentery infections. d'Herelle did nearly all of the early definitive work on the subject and summarized it in a book, published in 1921 and entitled *Bacteriophage; its Role in Immunity*. At this time the arguments raged over whether bacteriophage was an organism or whether it was something preformed in the cell, and on this point d'Herelle took what was eventually to be accepted as the correct view. He had, however, an overwhelming conviction that bacteriophages were powerful therapeutic agents, and a large part of his and of the general effort in phage research between 1920 and 1930 was spent testing phages against all sorts of diseases ranging from dysentery to tuberculosis.

Meanwhile, however, the scope of phage research was broadened with the description of many new phage species, which affected a wide variety of bacteria, and the generality of the phenomenon was amply documented but the central problems were postponed through a diffusion of effort. In the early 'thirties a number of able investigators studied phage for brief periods, and often with interesting results, but none of them continued with the subject. It is of interest that, even by this time, very few investigators had made bacteriophage the subject of their life work.

A turning-point came in 1936 when Delbruck and Luria, in a joint effort, initiated some phage studies in which the governing motivation was to understand the phenomenon of bacteriophage rather than to develop practical applications. This was by no means the first such study, but it was the first which led directly to other similarly organized efforts on what proved to be a rapidly expanding front.

The results were fruitful, the developments were rapid, and it is worth while trying to understand the reasons for the marked success of this approach. The scientists who participated were well trained in a variety of disciplines and very few came directly from medicine. There were physicists, biochemists, cytologists, geneticists, microbiologists, electron microscopists, and others. They had a high degree of internal discipline and by common consent they concentrated their attention on a small group of viruses with a common host. The importance of accurate quantitative results was stressed. Infection was studied at the cellular level. Scientific results were commonly exchanged before publication, and the published results have an accuracy and reproducibility which was often lacking from the earlier phage literature.

The success of this joint effort was immediate, especially in providing a number of organized and integrated findings, which was in marked contrast to the past, and the approach was so powerful that the disorganized effort in this field was swept away. Before long the influence of phage work extended beyond virology to biology in general, and to-day is of the greatest importance as a tool in the study of replication. There is, unfortunately, no time to consider this non-viral phase and we shall confine our attention to those aspects of bacterial virology which influence animal virology.

We left animal virology on the threshold of a new era. Within the past ten years new techniques have been changing the outlook of the animal virologist and there are definite signs that this branch of the science is developing along lines which break with the past. An attempt will be made briefly to assess the influence of one branch upon another at the methodological level, at the phenomenological level, and in terms of general scientific organization.

## Methodology

Methodology, although it sounds inherently dull, can be a crucial factor in the development of a science, and the introduction of the plaque technique by d'Herelle was certainly a turning-point for virology. The idea, derived from bacteriology, for the first time placed virology in a position to be studied quantitatively, in the sense in which that term is ordinarily used. The evaluation and refinement of this method was one of the first tasks carried out after the inception of the modern phage school.

Prior to 1952 the methods of quantitating the infectivity of animal viruses were really very rough, depending by and large on the death or survival of whole animals that had been given widely varying amounts of virus. There is no doubt that these and similar limitations greatly curtailed the development of the art. Therefore the introduction of the plaque method into animal virology by Dulbecco was a first-rate achievement. This advance was made possible by the recent introduction of tissue-culture techniques into virology. It is interesting and significant that the

method was introduced into animal virology by a student of phage and not an animal virologist.

In nine years the plaque method has been adapted to a wide variety of animal viruses, utilizing a variety of animal cells. This often reduced the errors of titration from several hundred to less than 15%. It provided a ready and precise way of obtaining pure line clones. Another major consequence of the use of the technique is the possibility of studying the events of infection at a cellular level, devoid of all the complications arising from using a whole animal. The study of the neutralization phenomenon is a good example.

Formerly there was no good way of allowing virus and antibody to react in a test-tube and then testing for, say, residual active virus without putting the whole mixture in an animal. After inoculation the investigator has little control and no knowledge of what goes on in the animal determining death or survival; for example, further reaction between antigen and antibody, dissociation of antigen and antibody, or slightly delayed infection with subsequent mobilization of the protective resources of the animal. These are all possible and each could affect the titration. In the new way, the antigen and antibody react in the test-tube, and then, after brief contact with the cells, the excess antibody and virus can be removed and the assay becomes relatively straightforward and uncomplicated.

The animal virologist now has the same basic tools as the bacterial virologist, although the animal-virus techniques are much more difficult than the corresponding bacterial ones. The host cells will not grow on simple media, and they will not grow into a monolayer overnight. The host cells do not tolerate handling very well, and the development of plaques often requires days. These technological difficulties are reasons for using the bacteriophage and its host as an experimental model.

## Phenomenology

In considering the different phases of the life-cycle of a virus the same subdivisions are useful for all viruses—adsorption, penetration, multiplication, maturation, and release—and these categories can be conveniently used for comparing the phenomenology of bacterial versus animal-virus infection. The adsorptive phase in each case requires electrolyte, and in both cases several different stages of adsorption may be demonstrable.

The penetration of viruses into cells appears to be one of the most varied functions among the major virus groups. The plant cell requires injury, apparently of a special sort, for infection to take place. For bacteriophage to breach the tough bacterial wall a complicated and specialized injection apparatus has developed, the existence of which has given rise to misgivings about the relationship of bacterial to animal viruses. Where this injection mechanism exists the virus coat is clearly left outside the bacterial cell, and this has definitely posed the query: Does the animal-virus coat get into the cell? Where the problem of animal-virus penetration has been considered at all, some form of phagocytosis or pinocytosis has been proposed as a likely mechanism, in which case the engulfment of the whole particle seems very probable. However, recent experiments with poliovirus (Joklik and Darnell, 1961) have suggested that some weakening of the coat may take place outside the cell. Clearly, experiments should be designed to determine the fate of the capsomeres with animal-virus penetration.

The absence of mature virus particles within the cell immediately after infection was first conclusively demonstrated with bacteriophage, and the reasons for this eclipse phase were quickly discerned as being due to separation of constituent virus parts on penetration and formation of new virus by assembly of several different constituents, possibly made in different parts of the cell.

This work inspired a number of experiments with animal viruses in which the occurrence of an eclipse phase was studied. The results were fairly conclusive with some viruses, leaving no doubt that, at least after penetration, a cell may contain no demonstrable mature virus particles, and the assumption that reproduction can proceed from a stripped virus core has been bolstered by experiments with naked viral ribonucleic acid (R.N.A.).

Maturation by assembly seems to be a hallmark of virus reproduction, and the mechanism of this assembly process has been worked out with T2 and T4. The non-specificity of assembly gives rise to a phenomenon called "phenotypic mixing," where particles are made up of parts arising from different parents. This non-specificity of assembly was also discovered with influenza viruses, but the interpretation of the results as being due to phenotypic mixing came directly from phage work.

Genetic recombination of viruses in mixedly infected cells provides still another example of a phenomenon worked out with bacteriophage which stimulated similar though necessarily much more restricted research with influenza and vaccinia. The same may be said for multiplicity reactivation and cross-reactivation, the salt requirements for virus adsorption, and the phenomenon of cell-killing by viruses. In many of these cases the behavioural similarities of the two different virus groups are striking. In some other areas, such as the changes in cell metabolism brought about by infection, the results with mammalian cells are quite different from those found with infected bacteria. This is not surprising, and at least part of the difference is related to the very large size of mammalian cells and the relatively small part of the cellular metabolism which the synthesis of virus requires. It is also probably due to the inherently different type of metabolism in animal cells.

### Latent Infections

Perhaps the most important effects of phage research on general virology may occur in the future, and this may be in the area of latent infections. Certainly the most fascinating part of the phage story concerns the nature of the lysogenic state. An invading virus becoming an integral part of the host cell genome, a latent virus replicating synchronously with the cell itself, and an invading virus altering the host cell phenotype were all fascinating and unexpected findings which provide a concept of parasite and host as evolutionary partners. The results suggest the possibility of a viral origin of at least part of the host genome and, conversely, mutation in the host genome as a possible mode of origin of the virus. The question is: Do any of these same phenomena occur with cells of higher organisms?

The possibilities are intriguing from many points of view. There are a great many viruses which remain

latent in higher organisms, and at the moment the mechanism of their maintaining a latent condition is completely unknown. "Latent" or chronic infections of mammalian cells *in vitro* have been described and studied in detail in recent years, but in most of these systems it is doubtful if the mechanism of virus survival is the same as in nature. Experiments in which both the host cell and the virus are irradiated have suggested, with bacterial viruses, that some homologies exist between virus genome and host genome. This has inspired similar attempts with irradiation of animal viruses and their host cells, but the results are inconclusive. However, experiments with tumour viruses have shown that these agents may multiply within a cell without killing it, and in some cases it is possible, though unproved as yet, that new cell antigens are produced as the result of infection (Habel, 1962).

It thus appears that no very definite case has been made out so far for anything corresponding to lysogeny with animal viruses. The important thing to note is that the bacterial work has stimulated a great deal of interest in the concept of this kind of parasitism at the genetic level, and the failure to confirm the similarities is probably mainly an indication that the state of the art of handling this type of virus and host material is not yet sufficiently advanced to answer such questions. Burnet suggested in 1931, on the basis of some experiments in lysogeny, that the lysogenic virus might be an integral part of the host genome, and, while this surmise was essentially correct, it required 20 years and the development of bacterial and virus genetics to prove it.

## General Organization

At the third level, that of general organization, animal virology is still in an undeveloped state. However, it is already apparent that it is becoming more removed from its close ties to medicine and is beginning to develop from within. This certainly augurs well for a fruitful development, and it may be hoped that it may proceed by a logical progression rather than in the hit-or-miss way characteristic of much medical research. Workers of very diverse training are moving into this area. At the moment a wide variety of animal viruses of academic interest are under study, and it is conceivable that, with time, the workers in this field may concentrate on a few key types, in a move comparable to the concentration on the T coliphages.

## Personal Experimental Work

Before finishing, I should like briefly to present some work being carried on currently in my laboratory with the collaboration of Dr. Robert W. Simpson (Simpson and Hirst, 1961). The excuse for including it here is that it furnishes a concrete example of the interdisciplinary forces that have been discussed. I mention this work with some diffidence, however, because it has not been developed very far and, at the present time, permits of no very far-reaching conclusions.

For the genetic analysis of a species genetic interaction between two members of the species is required. It was surprising to hear, just a short time after the birth of bacterial genetics, that genetic recombination takes place between two phage particles invading the same cell. Shortly thereafter Burnet described influenza recombination. Phage genetics turned bacterial virology into one of the central sciences of modern biology, while the influenza story which we are to follow has slowed to an almost imperceptible pace.

The basic experiment, which is due to Burnet and his collaborators, consists of crossing two related but antigenically different influenza A strains by putting both into either mouse brain or the allantoic sac and examining the viral progeny. These two strains may be considered to have M and W as antigenic markers. The W strain was virulent for mice by the intracerebral route while the M strain was not. After mixed infection it was possible to sort out four virus types from the progeny, the two parent viruses and two recombinant types, avirulent W and virulent M. A few other recombinants were shown in similar fashion over a period of years, and there is no doubt that these results were due to recombination of genetic material between influenza viruses.

Fraser (1959) confirmed and elaborated on this experiment, as shown diagrammatically in Table I, by crossing these same strains, isolating a number of M strains each time, testing them for virulence and backcrossing them with the virulent W strain. Fraser showed

TABLE I.—*Recombination of Virulence*

(1) NWS++++×M−→Mostly M−, M+, very few M++, etc. (also NWS+++, NWS++, etc.)

(2) M+(from reaction 1)×NWS++++→M+, M++, and a few M+++, etc.

(3) M++(from reaction 2)×NWS++++→M++, M+++, and some M++++

(4) M+++(from reaction 3)×NWS++++→M+++ and M++++

A schematic representation of how Fraser (1959) obtained fully virulent M virus (M+++) from completely avirulent M virus (M−) by crossing M− with NWS++++ (maximum virulence). Some M progeny of each cross showed a moderately enchanced virulence over the M parent type. By successive backcrossings with NWS, fully virulent Melbourne (M++++) was obtained.

by this method that strain M went from avirulence to full virulence by a series of small steps, and he thus conclusively demonstrated that this kind of virulence is multigenic in origin and therefore an unsuitable marker for beginning the analysis of a complicated system.

It will not be profitable to review all of the developments which came out of this attack on the problem, some of them from our own laboratory. The main defect of this approach was that it could not be made really quantitative, and quantitation has always been an essential part of genetics. After a few years this work was dropped and there have been virtually no publications in this field for the past four or five years.

At about this time we began looking for an R.N.A. virus which could be handled quantitatively and was suitable for recombination studies. Newcastle disease virus seemed like a good possibility and was studied extensively in our laboratory by Granoff (1959). In spite of good markers and a good quantitative assay system, no evidence of recombination could be found. The reasons for this failure have remained obscure.

Poliovirus, which also lends itself well to quantitative work, was tried and, actually, a low frequency of recombination was obtained in a cross of two mutants, each of which had become resistant to a specific plaque-forming inhibitor, one in a bovine and one in a horse serum. This type of approach is being further pursued, but progress has been slow and some of the difficulties in obtaining suitable markers have been described by Dulbecco (1961).

Influenza, as mentioned above, gives readily demonstrable recombination, even in crude systems, but it has been very difficult to develop quantitative methods. As we have already indicated, an essential step for good quantitation for viruses is a plaque-forming system, and it seemed unfortunate that most strains of influenza A either do not kill the cells in monolayers in such a way that plaques can be detected or else the efficiency of plaque formation is so low as to make the technique virtually unusable.

Most strains of influenza are rather strictly pneumotropic in the mouse, but a mutant of the original human influenza strain (WS) was isolated many years ago which has the capacity of multiplying in the endothelial cells of the mouse, and, as a consequence, it can multiply in the brain, where it is lethal. This strain (WSN), inaccurately described as being neurotropic, also produces excellent sharply outlined plaques on chick fibroblast monolayers, and does so with a very high efficiency. As noted before, influenza strains in general either produce poor plaques at low efficiency on this medium or fail to produce plaques at all.

Using this plaque-forming strain, we have developed a system in which plaque formation is used as a selective marker for those particles which have undergone recombination. Selection is an essential feature of recombinational systems where the amount of nucleic acid per particle is so small as to make recombination a very infrequent event. The fact that all recombinant clones are efficient plaque-formers of course enhances the possibility of working with them on a quantitative basis.

The method is carried out as follows: The so-called neurotropic plaque-forming strain (WSN) (or a recombinant derivative of it) is irradiated by ultraviolet light until very little survives, and it is then used to "infect" suspended chick fibroblasts along with another active non-plaque-forming (or weak plaque-forming) strain. These cells, which have received at least one of each type of particle, are then plated as infective centres on fibroblast monolayers and overlaid with agar.

If the suspended cells are infected with either of these two types of virus singly, no plaques will be formed and the controls are therefore negative. After combined infection, however, a number of clearly outlined plaques appear. These plaques are readily transmissible in series, and the virus in them is plentiful and plates with high efficiency. In the case of some crosses the new plaques are caused by viruses of the same serotype as the non-plaque-forming parent.

Similar experiments have been carried out using a number of influenza A strains, and we have been successful in isolating plaque-forming recombinants from a wide variety of them, including old laboratory strains isolated from epidemics in the early 'thirties as well as those obtained from Asian influenza. The actual frequency of recombination is not known and is difficult to determine from this type of approach. All that can be said is that the rate varies enormously with different crosses and may bear some relationship to the similarities of the strains being crossed. Strains that were very dissimilar antigenically, such as types A and B, gave no recombination.

In order to see whether this system was truly selective, plaque-type recombinants were examined for other characters. A cross was carried out between a Melbourne strain (isolated in 1934 and converted to plaque formation by recombination) and the prototype of A2 or Asian influenza, isolated in Japan in 1957. These two strains are quite disparate in a number of respects, although both are influenza A. Eighty-six plaque-forming recombinants were tested for five markers, such as the capacity to infect, position in the receptor gradient, and heat stability. A very high rate of recombination was found for these markers. More surprisingly, these five markers appeared to assort independently, as indicated in Table II, which shows that a large number of the possible combinations of recombinant characters was actually found. For at least two passages these new characters proved to be stable.

TABLE II.—*Distribution of Markers Among Progeny of a Cross Between Plaque-forming M (UV) and Non-plaque-forming J Strains*

| J Parent Type | Phenotypic Classification of Virus for: | | | | | No. of Clones Found |
|---|---|---|---|---|---|---|
| | Heat Resistance | Spontaneous Elution | Agglutination of RDE-treated Cells | Rabbit Serum Inhibition | Infectivity | |
| J progeny recombinant for plaque formation | − | − | − | − | − | 3 |
| | − | − | + | − | − | 1 |
| | − | + | − | − | + | 9 |
| | − | + | + | + | + | 3 |
| | − | + | + | − | + | 10 |
| | − | + | + | + | − | 2 |
| | − | + | + | − | − | 16 |
| | + | − | − | − | − | 4 |
| | + | − | + | − | + | 1 |
| | + | + | − | − | + | 4 |
| | + | + | − | − | − | 1 |
| | + | + | + | − | + | 6 |
| | + | + | + | + | − | 7 |
| | + | + | + | − | − | 19 |
| Total recombinants .. | 42 | 77 | 65 | 12 | 33 | 86 |

86 J type recombinants were analysed for the five characters shown. + Indicates that the character was from the M parent (recombinant); − indicates J parent type (non-recombinant).

From these preliminary results we feel that it may be possible to analyse the genetic system of the influenza A viruses in a systematic manner along classical lines. If so, it will be the first time that a genetic system based solely on inheritance through R.N.A. has been so examined. Certainly, if the genetics of influenza virus inheritance can be worked out it will strengthen our knowledge of this very interesting and important organism in such a way that we shall be much better able to cope with the practical problems which influenza presents in the form of its apparently great mutability with widely swinging variations in virulence for man. On the other hand, this may be only a step in the direction of analysing other animal viruses, which we are not prepared to work with at the present time.

## Conclusion

We return now to our point of entry, and we have seen enough, I hope, to realize that the nature of virology as a biological science cannot be expressed in a word or in a phrase, or perhaps not precisely at all. If we must characterize it further, we can say that it is not a taxonomic science like ornithology because very likely there may be no direct relationship between many viruses in an evolutionary sense. To say that the ties that unite virology are methodological is certainly true, but only partly true, and one has the feeling that the unity must transcend mere methods. Perhaps cytology provides a closer analogy than any other biological science. In any case, viruses as subcellular reproducing agents occupy a niche whose relationship to other

biological entities remains to be discovered. At the present time the outstanding similarities are morphological and the evolutionary similarities or differences remain to be worked out.

In closing, I should like once again to refer to the Institute. It was obviously in the spirit of fostering a broad academic approach that the Institute of Virology was founded here at the University of Glasgow, and, as I have tried to indicate, the creation of this organization with such adequate housing and equipment, but much more importantly with such able leadership, was a wise and timely move and should be the basis for self-congratulation on the part of the University. It is certain that the organization will grow with the times, and, as I have indicated, the times seem most propitious.


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# SOURCES AND SEQUELAE OF SURGICAL SEPSIS*


BY

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The problem of infection in surgical wounds has always been a contentious one. Lord Lister certainly found it so. From time immemorial the surgeon's attention has, naturally enough, been mainly focused on the drama and challenge of operation, but in recent years he has recognized that the pre-operative preparation and immediate post-operative care of the patient are so integral a part of the operative procedure that the operator himself must assume major responsibility for them. But, in general, the surgeon has been disinclined to concern himself seriously about the "occasional" infected wound. It is rarely lethal, it usually resolves ultimately, complications such as wound herniation may not develop for ages and may then be treated by someone else, and, in any case, it is best handled by his registrar or house-surgeon.

Indisputable evidence exists in all parts of the world that infection of surgical wounds is occurring in either the operating-theatre or wards, that the organism mainly concerned is the *Staphylococcus aureus*, that the organisms are being born and bred in the hospitals, and that the likelihood is that they will increase in both frequency and possibly virulence. The surgeon's reaction to this has often been mostly one of bland indifference; some surgeons flatly deny that any of their wounds ever become infected. Others admit that sometimes they have a little trouble, but never anything like the horrors that are reported from the professorial unit where this problem is being investigated. But, of course, you have to expect some rather odd things from a professorial unit.

Our interest in this work started some four years ago when we instituted a purely clinical survey of clean surgical wounds. Some 764 of them were carefully scrutinized. They were all uncontaminated wounds which should have healed by first intention, but 8% of them showed clinical infection (B. P. Morgan, 1958, personal communication).

## Findings of a Survey

More recently a much more intensive survey was undertaken to try to identify the source of the infection. Over a six-months period patients and staff were systematically and intensively investigated clinically and bacteriologically. In brief, our findings (Rountree *et al.*, 1960) were:

(1) In spite of poor and antiquated operating-theatre facilities with inadequate ventilation there was little evidence that wound infection was initiated in the theatres. During 25 operations 167 nasal swabs were taken from 67 people. In 46% *Staph. aureus* was isolated. Of these 67 people, 42% were carriers on at least one occasion. It is of interest that during the 25 operations the people in the theatre comprised:

| | |
|---|---|
| Surgeons, anaesthetists, visiting doctors .. .. | 21 |
| Nursing sisters .. .. .. .. .. | 14 |
| Student nurses .. .. .. .. .. | 16 |
| Medical students .. .. .. .. .. | 10 |
| Instrument and *x*-ray attendants .. .. .. | 6 |

The "permanent" members of the surgical team numbered nine. Of these nine, six were permanent nasal carriers. However, no patient was apparently infected in the theatre with any of these strains, and it would seem that few of these carriers were dispersers of their organisms, and possibly their strain was not highly virulent. But the potentialities of this situation are considerable under different carrier circumstances.

(2) A total of 217 wounds were intimately examined. "Clean" wounds were those in which no possible reason for contamination was present. A cross-infection rate of 14% occurred in "clean" wounds and 68% in "dirty" wounds (Table I).

TABLE I.—*Cross-infection—"Clean" and "Dirty" Wounds*

| Wound | No. of Cases | Uninfected | Infected |
|---|---|---|---|
| Clean .. | 198 | 171 | 27 (14%) |
| Dirty .. | 19 | 6 | 13 (68%) |
| Total .. | 217 | 177 | 40 (18%) |

(3) The site of the wound on the body or the sex of the patient did not significantly affect the infection rate, nor was it influenced greatly by the surgeon. However, the type of dressing did. Gauze pads which absorbed blood, serum, or sweat and required early post-operative changing were associated with a much higher incidence of wound infection than when a spray-on plastic seal ("healex" or "nobecutane") was used. While 61% of wounds were infected under a gauze pad, only 7% were when sealed (Table II).

(4) Of 40 wounds showing cross-infection, 35 (87%) were infected with *Staph. aureus*, either alone or with other organisms. The predominant staphylococcus present in 60% of wounds was phage type 47, which is resistant to penicillin, streptomycin, tetracyclines, and usually chloramphenicol and

*Based on a paper read in the Section of Surgery at the Annual Meeting of the British Medical Association, Auckland, 1961.